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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Ames et al.

Group Art Unit: 1614

Serial No. 10/038,135

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Examiner: Jones, D.

Attorney Docket No. B00-001-2

For: Primary N-hydroxylamines

## DECLARATION UNDER 37CFR1.132

I, Bruce Ames declare and state as follows:

1. I am a Professor of Biochemistry and Molecular Biology, University of California, Berkeley, and a Senior Scientist at Children's Hospital Oakland Research Institute (CHORI). The University of California is the assignee of this invention, and I am an inventor. I am a member of the National Academy of Sciences and served their Commission on Life Sciences. I have been a member of the board of directors of the National Cancer Institute, the National Cancer Advisory Board. I have received the General Motors Cancer Research Foundation Prize (1983), the Tyler Environmental Prize (1985), the Gold Medal Award of the American Institute of Chemists (1991), the Glenn Foundation Award of the Gerontological Society of America (1992), the Lovelace Institutes Award for Excellence in Environmental Health Research (1995), the Honda Prize of the Honda Foundation, Japan (1996), the Japan Prize, (1997), the Kehoe Award, American College of Occup. and Environ. Med. (1997), the Medal of the City of Paris (1998), the U.S. National Medal of Science (1998), The Linus Pauling Institute Prize for Health Research (2001), and the American Society for Microbiology Lifetime Achievement Award (2001). I have over 450 publications am among the few hundred most-cited scientists (in all fields):23rd most-cited (1973-1984). My curriculum vitae is viewable at [www.bruceames.org](http://www.bruceames.org).

2. Krishna et al. (1998, Journal of Medicinal Chemistry 41(18):3477-92) studied the effect of ring size, oxidation state and redox midpoint potentials of five or six-membered secondary nitroxides historically used as biophysical probes. The intermediate reduced forms of Krishna's

nitroxides are the corresponding five or six-membered secondary hydroxylamines (e.g. compounds 1b, 2b, 5b, 6b, 9b, 11b-17b, 19b, 22b, 23b, 25b-27b, 29b, 36b-38b, 40b, 42b, 48b, 52b, 53b, and 55b; Krishna (1998) p.3478, col.2, lines 30-32).

Our claims require primary N-hydroxylamines, which are structurally and functionally different from the cyclic secondary hydroxylamines studied by Krishna, especially as they relate to biological systems. By functionalizing a second proton, particularly in a cyclical carbon ring, cyclic secondary amines present substantially different chemical reactivities, in part by reducing the availability (reactivity or nucleophilicity) of the free electron pair of the Nitrogen. This can be seen, for example, in the strikingly different redox potentials of secondary and primary hydroxylamines. Primary hydroxylamines have redox potentials in the 300 mV range (see Fig.3 of Tamilmani et al., 2003, DuPont Electronic Technology, <http://www.ekctech.com/images/feature-stories/MRS-Interaction%20between%20ceria%20and%20Hydroxylamine.pdf>, of record), near that of the


intracellular reducing potential (e.g. Sies, et al., 1977, Euro J Biochem 72, 301-7, abstract of record), whereas the cyclic secondary hydroxylamines of Krishna et al. provide redox potentials ranging from 722 to 960 mV (see, e.g. Krishna et al., 1992, PNAS USA 89, 5537-41, of record).

Krishna (1998) describes use of dozens of different compounds, but every one is a similar cyclic secondary nitroxide (and the corresponding hydroxylamines and amines). Krishna (1998) provides no suggestion or motivation to deviate from his teachings and employ a structurally and functionally distinct class of hydroxylamines, particularly since Krishna (1998) repeatedly reports that variation in redox potential across his reagents showed no significant correlation between protection and redox potentials (e.g. Krishna, 1998, at p.3488, col.2, line 41-45).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date:

6/11/04



Prof. Bruce Ames